NeoBiota 59: 99–118 (2020)
doi: 10.3897/neobiota.59.51906
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Effects of a recalcitrant understory fern layer in an enclosed tropical restoration experiment

Luis C. Beltrán[1], Karla María Aguilar-Dorantes[2], Henry F. Howe[1]

1 *Department of Biological Sciences (m/c 066), University of Illinois at Chicago, Chicago IL 60607, USA*
2 *Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos, C.P. 62209, México*

Corresponding author: Luis C. Beltrán (lbeltr6@uic.edu)

Academic editor: Elizabeth Wandrag | Received 11 March 2020 | Accepted 9 July 2020 | Published 30 July 2020

Citation: Beltrán LC, Aguilar-Dorantes KM, Howe HF (2020) Effects of a recalcitrant understory fern layer in an enclosed tropical restoration experiment. NeoBiota 59: 99–118. https://doi.org/10.3897/neobiota.59.51906

Abstract

Establishing mixed-species tree plantings and fencing them to protect seedlings from herbivory is a valuable strategy for reconnecting forest fragments separated by agropastoral lands. However, fencing may provide exotic plants with the escape from herbivory required to invade the understory of planted communities. Here we take advantage of such a situation to ask how the identity of planted species and the resulting canopy cover influenced invasion success by the Asian swordtail fern (*Nephrolepis brownii* Desv. Nephrolepidaceae) in a 13-year-old tropical restoration experiment. Through a seed addition experiment, we also evaluated the effects the ferns had on recruiting seedlings. We found that the invasion was most acute in the unplanted control plots where canopy cover was consistently scarce. Frond density correlated negatively with canopy cover, though most of the variance in the model is explained by the design of our experiment (r^2m = .161, r^2c = .460). Between planting treatments that differed in the dispersal mode of the planted trees, the wind-dispersed treatment had higher fern density and longer fronds than the animal-dispersed treatment. The animal-dispersed treatment had the highest recruiting species richness, which was negatively correlated with fern density (r^2 = .748). The seed addition experiment confirmed that mortality rates increased where frond density was higher ($F_{1,41}$ = 7.159, p = .011) and germination rates were lowered for the smaller-seeded species ($F_{1,42}$ = 13.2, p = .002). To prevent recalcitrant understory layers from establishing in plantings in the future, we recommend: (1) establishing larger plantings or expanding existing ones to minimize edge effects (particularly light filtration), (2) supplementing young plantings with additional seedlings to prevent canopy gaps from forming, and (3) planting an assemblage of species that cover the full forest strata and have consistently full tree-canopies.

Keywords

Edge effects, invasive species, Los Tuxtlas Biosphere Reserve, *Nephrolepis brownii*, tropical restoration plantings, Veracruz

Copyright *Luis C. Beltrán et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Mixed-species plantings are established to enhance the natural succession of forests in degraded lands (Parrotta and Knowles 2001; Lamb et al. 2005; de la Peña-Domene et al. 2013). These plantings can be used to reconnect forest fragments as a stepping-stone biological corridor, a design that provides greater flexibility of land use for local landowners than continuous biological corridors do (Rey Benayas et al. 2008). For example, the space in between plantings can be used as pasture for cattle, so long as the plantings are fenced to prevent cattle from devouring recruiting seedlings (Holl and Quiros-Nietzen 1999; Aide et al. 2000). However, fences will protect plants indiscriminately, including unwanted exotics. Under favorable light conditions, release from herbivory can enable exotic plant species to act as aggressive invaders, altering the structure of the understory and potentially the fate of the restored patches (Keane and Crawley 2002).

Invasive plant species can arrest succession by dominating the plant understory through resource competition and allelopathy, resisting displacement by native species for an extended period of time (Young and Peffer 2010). A dense understory can also benefit small seed-eating mammals such as rodents, elevating the rate of seed predation under the invaders' canopy (den Ouden 2000). An understory characterized by such pervasive disruptors of succession is known as a 'recalcitrant understory layer' (Royo and Carson 2006). Species capable of forming this layer typically have three key characteristics: (1) rapid vegetative growth (e.g., often spreading through rhizomes), (2) reduced susceptibility to elimination, and (3) have long-living genets (Young and Peffer 2010). Eliminating a recalcitrant understory layer is possible when a dense tree canopy cover has already been established, but this is time-consuming and labor-intensive (Hill and Silander 2001; Douterlungne et al. 2010).

Ferns provide multiple examples of recalcitrant understory layers (Horsley 1993; George and Bazzaz 1999a, b; Marrs et al. 2008). Their ability to suppress seed germination is supplemented by slow decomposition of fern litter that tends to accumulate under fern thickets (George and Bazzaz 1999b; Allison and Vitousek 2004). One fern species with potential for establishing recalcitrant understory layers is the Asian sword-tail (*Nephrolepis brownii* Desv.) (Hovenkamp and Miyamoto 2005; Sharpe and Shiels 2014). It is naturalized in the tropical Americas where it is considered an invasive species with important economic and ecological implications (Proctor 1989; Robinson et al. 2010; CABI 2019). Where established, it is common from sea level to 1700 m in forests, although it is most common in open vegetation (e.g., roadsides, riverbanks, open thickets). This species' ability to arrest succession has not been evaluated, despite its continued presence in some tropical restoration plantings in Veracruz, Mexico.

Here we use the presence of *N. brownii* in our 13-year-old restoration planting experiment in tropical Mexico to assess (1) the invasibility of these plantings and (2) the effect that *N. brownii* has on recruiting seedlings. For the former, we evaluate the extent of the invasion using frond density (m^{-2}) by restoration treatment and in response to canopy cover. The restoration treatments include two planting treatments that differ in the dispersal mode of the planted trees (wind-dispersal vs. animal-dispersal) and an unplanted control simulating natural succession. Canopy cover differs between plots

but is especially lacking in the control treatment. Given how species capable of forming recalcitrant understory layers typically benefit from lacking tree cover (Royo and Carson 2006), we predict that the ferns would be more numerous in the unplanted controls and in plantings with lower tree-canopy cover. As an additional assessment of invasive success, we use frond length measurements, predicting that they correlate with frond density. This hypothesis is based on previous studies that have shown reduced size-density tradeoffs for invasive plants, allowing them to dominate the understory (Jakobs et al. 2004; Royo and Carson 2006). We also predict that other disturbance-adapted species capable of dominating understory, such as exotic grasses and hemi-epiphytic lianas, will be more common in the unplanted control treatment.

To assess the effect *N. brownii* has on plant recruitment we compared recruited woody species richness in response to frond density. We predicted that higher frond density would result in lower species richness. To verify that this pattern was not purely correlative, we established a seed addition experiment under varying frond densities to evaluate seedling performance. We predicted that small-seeded tree species would be most affected by frond density, with lower germination, higher mortality, and lower biomass than larger-seeded species. Examining the invasibility of these plantings revealed design improvements that could prevent future plantings from developing a recalcitrant understory of *N. brownii*, and possibly of other invasive species.

Methods

Study area

The study was conducted in the Los Tuxtlas Biosphere Reserve (~3,300 km^2), in the state of Veracruz, Mexico. Mean annual rainfall in the region is 4,900 mm and temperature 27 °C. Dry seasons typically extend from March through May and rainy seasons from June to February (González-Soriano et al. 1997). Deforestation driven by the demand for cattle pasture has resulted in highly fragmented forests in the region (Mendoza et al. 2005). These fragments are home to +350 tree species, the majority of which are dispersed by animals (Ibarra-Manríquez et al. 2015).

Restoration Plantings Experiment

Restoration plantings were established in June 2006 to facilitate the movement of plants and animals in a permanent agricultural landscape. The rationale was to determine if planted animal-dispersed tree species accelerated tropical forest succession by attracting more dispersal agents that bring forest seeds with them than planted wind-dispersed stands or unplanted controls. The experiment consisted of 24 30 × 30 m fenced plots set on an 8 × 3 grid, each separated by 35 m of actively grazed pasture (central GPS point 18°35'43.64"N, 95°06'06.29"W). The plantings included three treatments: Eight plots were planted with seedlings of 12 native animal-dispersed spe-

cies (animal), eight with seedlings of 12 native wind-dispersed species (wind), and eight were left unplanted to simulate natural succession (control) (Fig. 1). Each plot is divided into four subplots of 13 × 13 m separated by a 4 m wide open corridor. Some planted species in the animal-dispersed treatment like *Ficus yoponensis* (Desv.), *Inga sinacae* (M. Sousa & Ibarra-Manr.), and *Stemmandenia donnel-smithii* (Rose) have been fruiting consistently since 2010. However, seed rain between planted treatments has not differed significantly (Popoca-Ortega 2016).

To date, seedlings of 78 species of trees that were not planted have recruited in experimental plots (unpublished data from 2019 census). For details on the experimental design, the study area, and early-recruitment patterns, see de la Peña-Domene et al. (2013).

Study species

Nephrolepis brownii is a terrestrial, sometimes epiphytic fern, native to Southeast Asia. This species is more drought-resistant than most ferns due in great part to its erect rhizomes (Hovenkamp and Miyamoto 2005). The capacity of *N. brownii* to spread through underground runners allows it to quickly exploit gaps in the canopy (Sharpe and Shiels 2014), a characteristic seen in other fern species capable of forming a recalci-

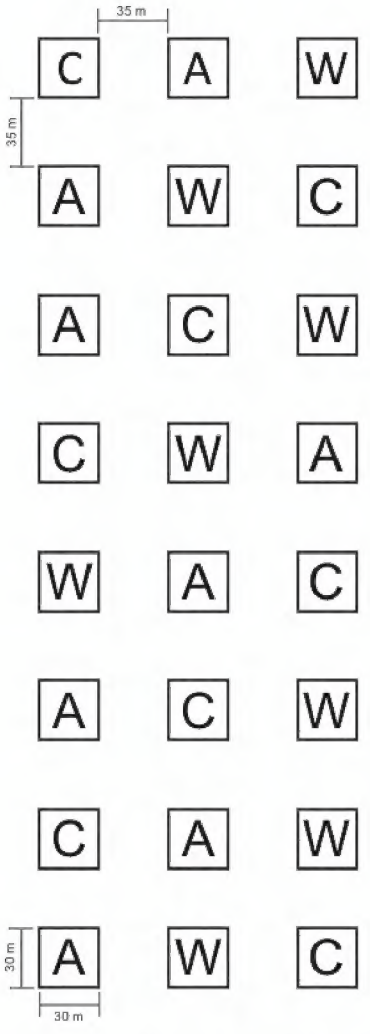

Figure 1. Experimental design of the 24 restoration plots belonging to three treatments: (A) plots planted with animal-dispersed trees, (W) plots planted with wind-dispersed trees, and (C) unplanted control plots. Adapted from de la Peña-Domene et al. (2013).

trant understory layer (Young and Peffer 2010). Aboveground, this species typically has 5 or 6 fronds per ramet, though the ramets themselves can be difficult to distinguish in the field. In this study, we use frond counts to estimate fern density. A full description of *N. brownii* is provided by Hovenkamp and Miyamoto (2005). Specimens collected from this study are deposited in the herbarium HUMO of the Center for Biodiversity and Conservation Research of the Autonomous University of the State of Morelos and the herbarium of the Biological Station of Los Tuxtlas.

Census of N. brownii and Recruiting Seedlings

To evaluate the extent of the invasion, we randomly established 4 1 m^2 quadrats (total of 384) within each subplot of every plot. To select quadrats, we used a random number generating code in RStudio to yield four numbers from 1 to 169, each number representing a possible quadrat in the 13 m^2 subplots. We counted the number of fronds within each quadrat. We also recorded the presence of grass or hemiepiphytic lianas in the quadrats as percent cover where present. This was done to account for other types of understory layers that might suppress tree recruitment. Quadrats were treated as "grass- or liana-dominated" when percent cover exceeded 50%. To obtain frond length measurements, we haphazardly selected five unfurled fronds per quadrat to measure. The recruiting plant community (> 10 cm tall woody plants) within the 1 m^2 quadrats and the trees creating canopy cover over the quadrat (recruits over 1.3 m tall) were recorded and identified to species. The latter was done to determine the frequency with which planted trees provided canopy cover over the recruiting seedlings and/or ferns. To estimate canopy cover, a photograph of the canopy covering each quadrat was taken from the middle of the quadrat at a height of ~ 1.3 m and analyzed using the environmental application for iOS devices, %Cover. The raw results of this study are available in Suppl. materials 4, 5.

Seed Addition Experiment

To determine whether fern cover affected the germination, growth, and survivorship of tree seedlings, we established a seed addition experiment within the restoration plots. Two late-successional, animal-dispersed tree species characteristic of the forest of Los Tuxtlas, *Brosimum alicastrum* (Sw.) and *Guarea grandifolia* (DC.), were selected for this study. Previous censuses showed that recruitment of these two species in the plantings were rare, which allowed us to more easily keep track of the fate of added seeds.

Seeds of animal-dispersed *B. alicastrum* (8–13 × 13–18 × 13–18 mm) and *G. grandifolia* (13–25 × 10–15 × 8–15 mm) were collected from 20 and 4 fruiting adults respectively in early-to-mid June of 2018 (seed dimensions from Ibarra-Manríquez et al. 2015). Seeds were cleared of remaining aril and wiped with 10% Ethanol before being dispersed to a randomly selected subplot within each plot in late June. Each selected subplot received 20 *B. alicastrum* and 12 *G. grandifolia* seeds spread out around four

equidistant points close to the center of the subplot to minimize competition between them. The specific location of the added seeds was marked with stake flags and seedlings were tagged with flagging tape as they germinated. Monthly censuses were carried out for a year to record germination, mortality, and seedling height. Seedlings were harvested in late June of 2019 to assess performance through biomass allocation: (1) total leaf count, (2) leaf dimensions (length and width), (3) leaf mass, (4) root mass, (5) shoot mass, (6) root-to-shoot ratio, and (7) total seedling mass. Mass measurements were taken after the seedlings were dried for 48 hours in a desiccating chamber at 40 °C. The raw results of this experiment are available in Suppl. material 6.

Statistical Analysis

Differences in average frond density were analyzed using a generalized mixed-effects linear model with a negative-binomial distribution where the nested design (quadrats within subplots within plots) was included as a random effect. Differences in frond length between treatments were analyzed using a mixed-effects linear model where each frond is the experimental unit and the random nested effect accounts for this additional level (fronds within quadrats within subplots within plots). To evaluate the relationship between frond density and frond length however, we used average frond length per quadrat to avoid pseudo-replication. Here too, we used a mixed-effects model with the nested design of the census at a quadrat scale included as a random effect. The relationship between frond density with canopy cover was also analyzed using a mixed-effects linear model with the same random nested effect as the previous model. An ANCOVA was used to determine if the relationship between frond density and canopy cover differed by treatment. Differences in recruiting species richness between treatments were analyzed using a generalized linear model with a Poisson distribution log link. Linear regressions were used to evaluate the effect of frond density on species richness. An ANCOVA was used to determine if the relationship between frond density and recruiting species richness varied by treatment. For the species richness models, data was pooled by plot in light of how smaller scales artificially limit the variable's range of values. Quadrats that were grass- or liana- dominated (> 50% cover) were excluded from models that used frond density or length as a variable.

To determine if *B. alicastrum* and *G. grandifolia* differed in their response to frond density, we compared the relationship between their biomass features and frond density using ANCOVA. Frond density in these cases corresponded to the averaged frond density of the subplot where the seeds had been sown. The analyses were carried out at a subplot scale to avoid pseudo-replication of frond density values and because quadrat measurements from the earlier census did not correspond to where seeds were sown. When the interaction was significant, we evaluated the effects of frond density using linear regressions for each species.

In January 2019, trespassers cut some of the germinated seedlings. Most of the cut seedlings survived, but all were excluded from the mortality and biomass features

ANCOVAs. All analyses were carried in RStudio ("R version 3.6.1 (2019-07-05)") using the lme4 package to fit linear and generalized mixed-effects models (Bates et al. 2015), the MuMin package to estimate marginal (r^2m, considers only variance of fixed effects) and conditional correlation coefficients (r^2c, takes both fixed and random effects into consideration) (Barton 2015), and the lmerTest package to obtain p-values and approximations for denominator degrees of freedom for the mixed-effects models (Kuznetsova et al. 2017). The sjPlot package (Lüdecke 2019) was used to create mixed-effects model summaries (Suppl. material 7).

Results

Extent of Invasion of N. brownii

Of the 384 1 m^2 quadrats established for the census, 306 (80%) were recorded with *N. brownii*. The degree of invasion follows a progression by treatment; the control treatment has the highest degree of fern infestation (94.53% of quadrats have ferns), followed by the wind-dispersed treatment (82.81%), and then the animal-dispersed treatment (71.88%) (Fig. 2). Pairwise comparisons reveal that the control quadrats have significantly higher frond density than the animal-dispersed treatment ($z = 3.417$, $p > .001$) and marginally higher frond density than the wind-dispersed treatment ($z = 1.729$, $p = .083$). Differences between planting treatments were marginally significant ($z = 1.743$, $p = .081$; Fig. 3A). The controls also had longer fronds than the animal- (ANOVA, $t = 7.056$, $p < .001$) and wind-dispersed stands ($t = 5.49$, $p < .001$). Differences between planted treatments were marginally significant ($t = 1.829$, $p = .068$; Fig. 3B). Frond density is positively correlated with average frond length ($F_{1,252.76} = 128.8$, $p > .001$, $r^2m = .327$, $r^2c = .444$, Fig. 4A) and negatively correlated with canopy cover. In the case of the latter, more of the variance in the model is accounted for when the nested design of the census is considered ($F_{1,311.29} = 33.665$, $p > .001$, $r^2m = .161$, $r^2c = .460$, Fig. 4B). The relationship between frond density and canopy cover does not differ between treatments (ANCOVA, $F_{2,351.65} = 1.126$, $p = .325$). Control plots had more quadrats dominated by grass or lianas (18/128) than did animal- (3/128) and wind-dispersed treatments (1/128).

Effect of Invasion on Recruitment

Within the quadrats established for the census, a total of 54 woody plant species recruited, the majority of them animal-dispersed and all of them native except for *Citrus* sp. Recruited species richness was lower in controls (average = 6.8) than either planting treatment, though only marginally to the wind-dispersed treatment (vs. animal: average = 10.5, $z = 2.533$, $p = .011$, *vs.* wind: average = 9.38, $z = 1.841$, $p = .066$). Differ-

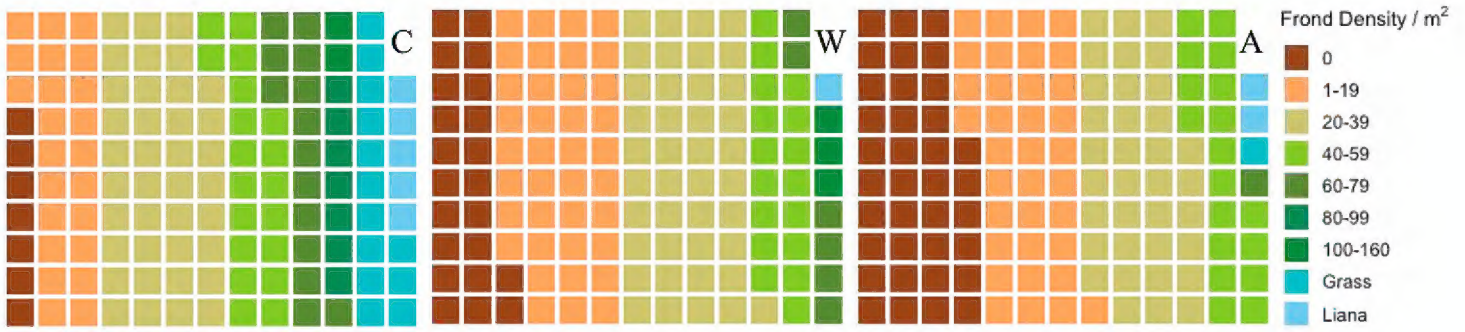

Figure 2. Ground cover of ferns, grasses, or lianas for the 384 1 m^2 quadrats excluding recruiting and planted forest plants. Each colored square represents a 1 m^2 quadrat in the (A) animal-dispersed, (W) wind-dispersed, or (C) unplanted control plots. Grass and liana quadrats were characterized as such when more than 50% of the ground cover was composed of grasses or lianas.

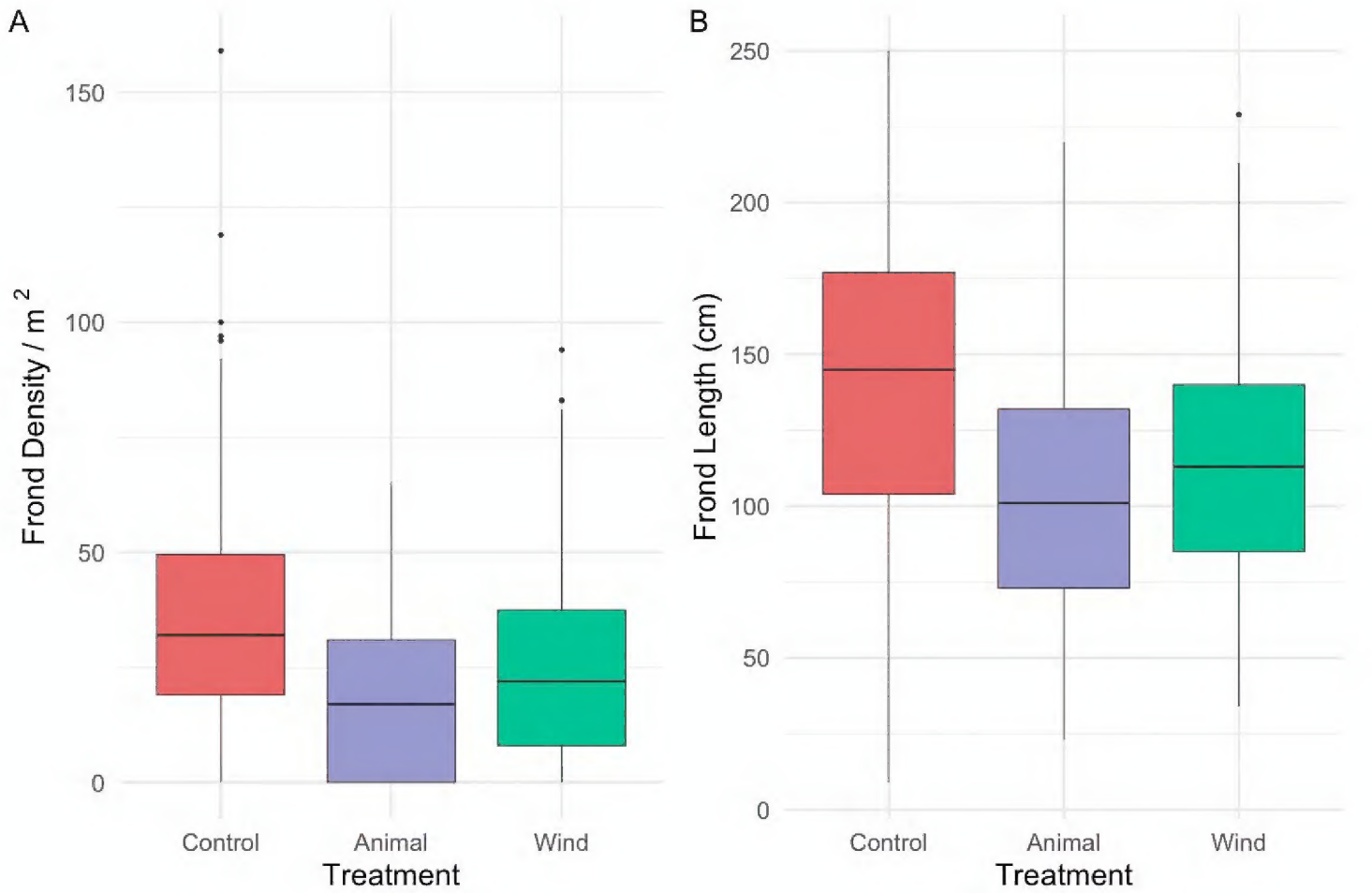

Figure 3. A boxplots of frond density (m^2) and **B** frond length (cm) by restoration treatment. Lower and upper box boundaries represent 25th and 75th percentiles, respectively. Lower and upper error lines represent Q1 – 1.5 * IQR and Q3 +1.5 * IQR, respectively. Middle band represents median values.

ences in richness between plantings were not statistically significant ($z = .713$, $p = .476$; Fig. 5A). The relationship between frond density and species richness varied marginally between treatments (ANCOVA, $F_{2,18} = 2.823$, $p = .086$). In the animal-dispersed treatment there was a strong, negative correlation between species richness and frond density ($F_{1,6} = 17.84$, $p = .006$, $r^2 = .748$). No significant relationships were observed between these variables in the wind-dispersed ($F_{1,6} = 2.224$, $p = .186$, $r^2 = .271$) and control treatments ($F_{1,6} = .929$, $p = .372$, $r^2 = .134$, Fig. 5B).

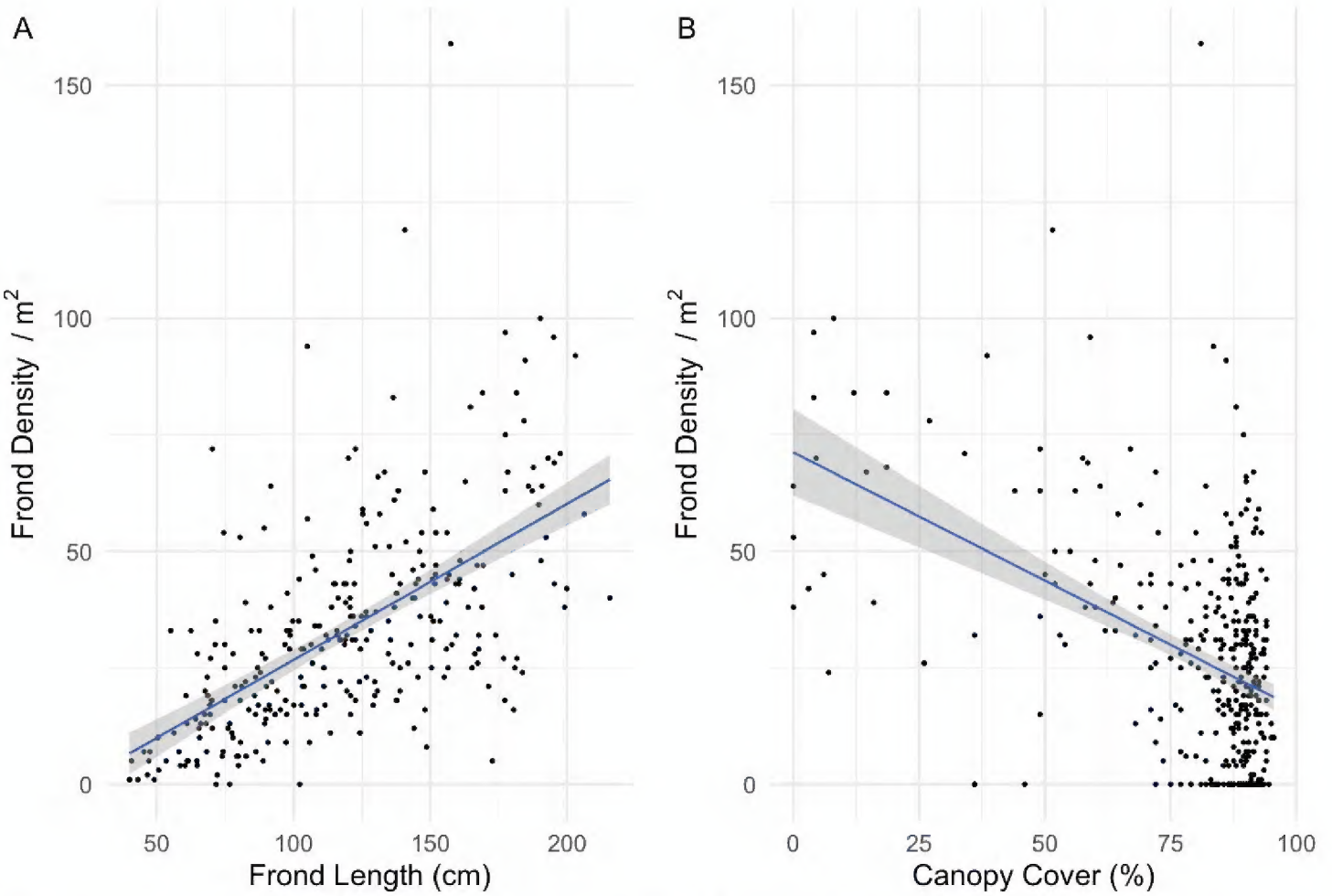

Figure 4. Relationship between frond density with **A** frond length and **B** percent canopy cover. Frond length values represent an average from five randomly selected fronds per quadrat. Band shades represent 95% confidence intervals.

Seed addition experiment

Frond density differentially influenced seedling performance by species (Fig. 6). The relationship between frond density and germination rates differed between *B. alicastrum* and *G. grandifolia* (ANCOVA, $F_{1,42} = 9.88$, $p = .003$). For *B. alicastrum*, germination rates were negatively correlated with frond density (linear regression, $F_{1,21} = 13.2$, $p = .002$, $r^2 = .386$) while *G. grandifolia* showed no trend (linear regression, $F_{1,21} = .484$, $p = .494$, $r^2 = .023$). Species also differed marginally in the seedling height response to frond density (ANCOVA, $F_{1,36} = 3.83$, $p = .058$). *Brosimum alicastrum* showed no trend (linear regression, $F_{1,17} = .862$, $p = .366$, $r^2 = .05$) while *G. grandifolia* showed taller seedlings as frond density increased (linear regression, $F_{1,21} = 5.424$, $p = .030$, $r^2 = .205$).

Frond density had marginally significant, negative effects on total leaf count ($F_{3,38} = 3.103$, $p = .086$, $r^2 = .196$). This pattern was similar for the two species (ANCOVA, $F_{3,38} = .731$, $p = .398$). Leaf length, leaf width, leaf mass, total seedling mass, shoot mass, and roots mass did not show trends in response to frond density ($p > .1$). However, root-to-shoot ratios did show a negative correlation with frond density for *G. grandifolia* (linear regression, $F_{1,21} = 5.06$, $p = .035$, $r^2 = .194$) that was not observed with *B. alicastrum* (linear regression, $F_{1,17} = .289$, $p = .548$, $r^2 = .017$). An ANCOVA confirmed that the species differ marginally in how frond density influenced their root-to-shoot ratios (ANCOVA, $F_{3,38} = 2.88$, $p = .098$).

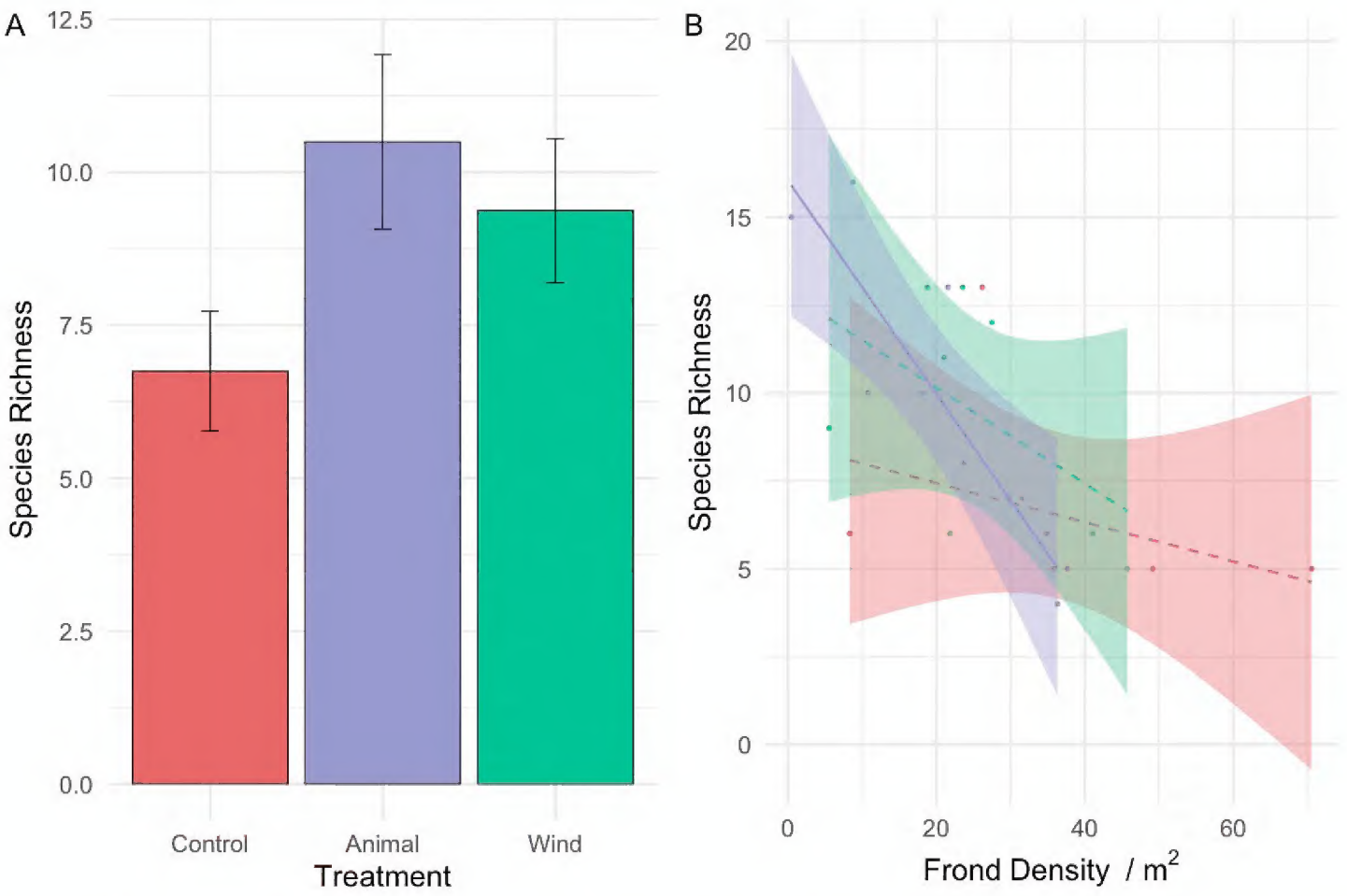

Figure 5. Species richness by **A** treatment and in response to **B** frond density at a plot scale. Error bars represent standard error. Band shade represents 95% confidence intervals. Dashed lines indicate non-significant correlations ($p > .1$). Solid lines indicate significant correlations ($p < .05$).

Mortality rates increased with increasing frond density ($F_{3,41} = 3.38$, $p < .001$). This pattern was similar for both species (ANCOVA, $F_{3,41} = .245$, $p = .624$), although *B. alicastrum* showed higher mortality rates than *G. grandifolia* ($F_{3,38} = 7.159$, $p = .011$). *Brosimum alicastrum* seedlings were also unable to survive past beyond 53 fronds per m^2 while *G. grandifolia* seedlings still survived in areas with over 80 fronds per m^2.

Discussion

Our results show that restoration treatments influenced the development of recalcitrant understory layers of *N. brownii*. Unplanted control plots meant to simulate natural succession were the most affected; this treatment had both the highest frond density and longest frond length. This clearly shows that low development of tree canopies enables invasion by exotic species released from herbivory. Effects of tree cover are also supported by the negative relationship between frond density and tree-canopy cover across treatments, which our model confirmed is largely explained by the design of our experiment. The importance of early development of a tree canopy layer is consistent with practices of the indigenous Lacandon Maya people of Chiapas, Mexico, who plant rapidly growing balsa (*Ochroma pyramidale* Cav. ex Lam. Urb.) to create a dense, wide canopy capable of suppressing the growth of another invasive fern species

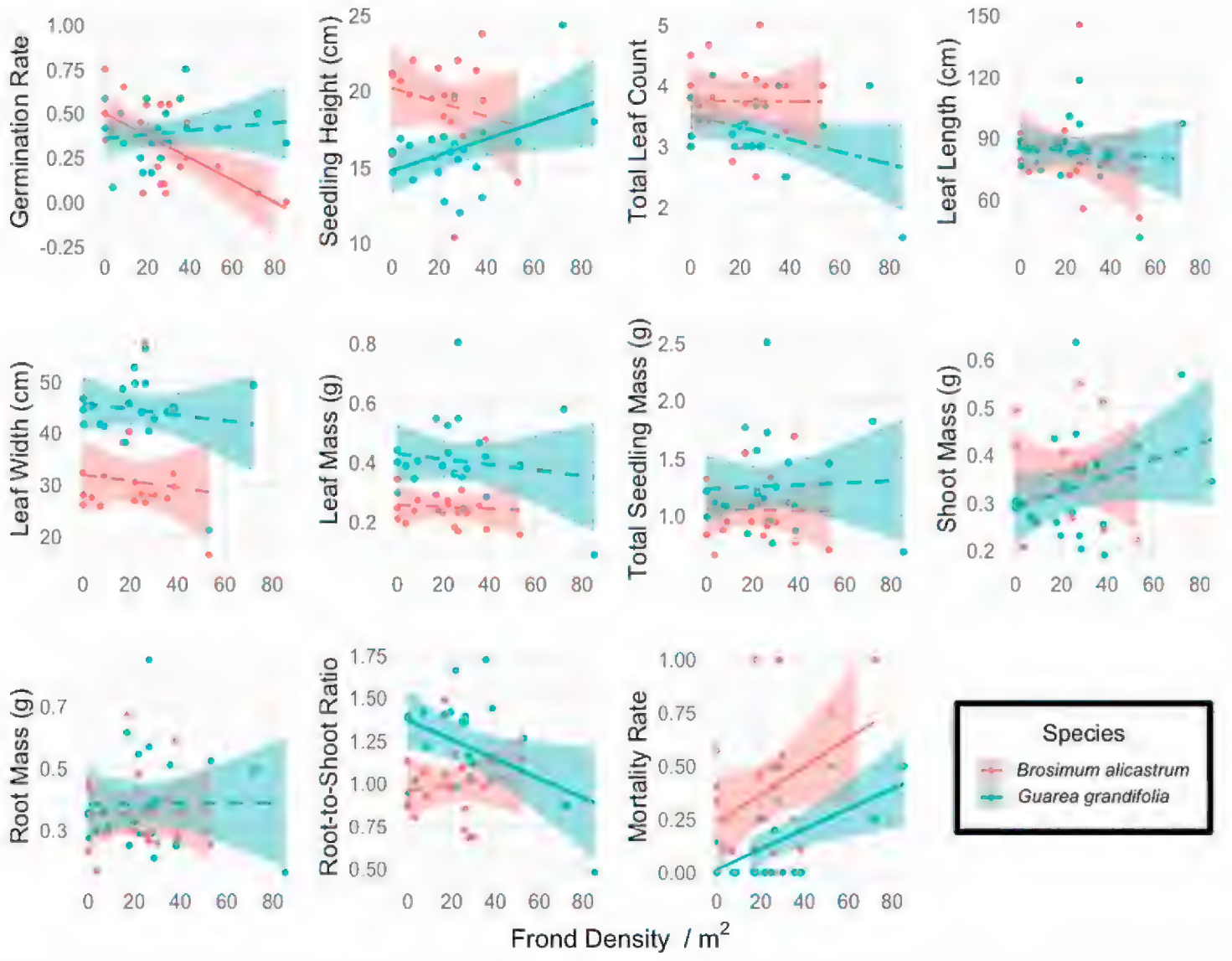

Figure 6. Seedling biomass responses of *Brosimum alicastrum* and *Guarea grandifolia* to frond density (m^2). Frond density values are the averages of each subplot's quadrat measurements where seeds were sown. Band shade represents 95% confidence intervals. Dashed lines indicate non-significant correlations ($p > .1$). Two-dashed lines indicate marginally significant correlations ($.05 < p < .1$). Solid lines indicate significant correlations ($p < .05$).

(*Pteridium aquilium*, Douterlungne et al. 2010; 2013). Balsa was one of the species we planted in the wind-dispersed treatment, but its low representation in the stands (1/12 of planted trees) likely limited its fern-suppressing attributes.

Differences between planting treatments provide additional insights. The animal-dispersed treatment had marginally lower frond density (average: 18.44/m^2) than the wind-dispersed treatment (average: 25.42/m^2). Though not as large a difference as that between either planting treatment or the control (average: 38.02/m^2), it suggests that the assemblage of planted species influences the invasibility of the plantings. One likely reason why the wind-dispersed treatment is more vulnerable could be the higher incidence of canopy gaps (Suppl. material 1). Considering this treatment has 1.3× more surviving planted trees, this is surprising (Suppl. material 2). However, when we determined the tree species that provided canopy cover over our quadrats, we found that relative to the animal-dispersed treatment, it was 1.2× less likely for planted trees to be part of the canopy cover in the wind-dispersed treatment (Suppl. material 1). In other

words, the boon the animal-dispersed treatment provides in curtailing the fern invasion is likely due to the growth patterns of the planted species that yield wider, more permanent canopies with multiple layers than the species we planted in wind-dispersed plots.

Differences between planted treatments are likely a byproduct of our experimental design; the species planted were chosen by dispersal mode and successional status (4 pioneers and 8 late-successionals) without focusing on the strata they filled in the forest or their phenology. As a consequence of this, the wind-dispersed treatment had one small tree species, three subcanopy species, and eight canopy tree species. In contrast, the animal-dispersed treatment is more balanced with three small, three subcanopy, and six canopy tree species (Suppl. material 3). Additionally, wind-dispersed species usually drop their leaves to maximize seed dispersal during the dry season, thereby enhancing light penetration for at least four months of a year (reviewed by van Schaik et al. 1993). Leaf drop is consistent with the increase in leaf-litter mass we detected in wind-dispersed plots (Valencia-Esquivel 2012).

Our results are consistent with the generalization that species selection influences the development of forest structure and the return of biodiversity (Parrotta and Knowles 2001; Sansevero et al. 2011; Longworth and Williamson 2018). We consider it important that future planting designs consider the strata that the assemblage of planted species will fill as they mature as well as their deciduous phenology and dispersal mode. The development of multiple, permanent or semi-permanent canopy layers will restrict the growth of recalcitrant understory layers. Furthermore, we suggest that practitioners prevent the development of canopy gaps by supplementing plantings with additional planted seedlings where necessary.

It is noteworthy that in our plantings, even a full canopy cover with multiple layers failed to prevent the invasion of *N. brownii*. This is likely due to the small size of the plots. At 30 × 30 m, these plantings experience adverse microclimatic conditions from edge effects known to alter forest structure (Magnago et al. 2015). The clearest way to minimize edge effects, including the light regimes that benefit invasives, is to maximize the size of the plantings (Laurance 2008).

Our survey of the plantings also confirmed that recruiting species richness is lower where frond density is higher, though this relationship was only statistically significant in the animal-dispersed treatment. The controls showed lower species richness than the planting treatments, likely because the unplanted plots are also subject to greater seed dispersal limitations that can explain the lower recruitment rates (Popoca-Ortega 2016). Nonetheless, results from the seed addition experiment strongly suggest that the relationship between frond density and species richness is a consequence of the adverse effects of fern cover on recruiting seedlings. The two sown seed species were both suppressed by fern invasion but performed differently in response to frond density. *Brosimum alicastrum* showed lower germination rates, and higher mortality rates in response to frond density while *G. grandifolia* showed greater shoot length, lower root-to-shoot ratio, and higher mortality rates. The difference in germination rates can be explained by differences in seed size between species. *Guarea grandifolia* produces seeds up to 3× larger than *B. alicastrum* (calculated from seed dimensions provided by

Ibarra-Manríquez et al. 2015). Greater seed mass provides germinating seedlings with more resources with which to grow in closed canopy conditions (Foster and Janson 1985). For *G. grandifolia*, resources are evidently invested in shoot length when the seedlings are covered by the understory canopy of *N. brownii*. This is reflected in the root-to-shoot ratios too; for *G. grandifolia*, the ratio increases with increasing frond density while *B. alicastrum* shows no pattern.

The differences these species exhibit in germination suggest that the tree species most likely to grow past the understory fern layer are those with greater seed mass. This is problematic in the context of restoration plantings because large-seeded species are less likely to be dispersed across inhospitable matrices (e.g., pastures) than small-seeded species (reviewed by Wunderle 1997; Beltran and Howe 2019). Even if these large seeds are dispersed to fern-infested plots, mortality rates are still higher where fronds are denser. Ultimately this means that invasion by *N. brownii* limits tree seedling recruitment and shapes the seedling community by favoring seeds large enough to germinate and grow past the understory but small enough to be dispersed to the plantings.

We do not observe *N. brownii* growing outside the plots as well as it does inside of them. This is likely because of the cows that graze the surrounding pasture; we have observed them eating the fronds that manage to grow past the outline of the fences. However, eliminating the fences in an attempt to suppress the ferns would terminate restoration; livestock eat tree seedlings as well as *N. brownii*. We have also not found *N. brownii* growing within the Los Tuxtlas primary forest, which is likely because of the deep shade cast by multiple layers of trees (some taller than 30 m) and/or endemic grazers that browse understory plants (e.g., *Tayasu pecari*, *Sylvilagus* spp.). One solution to restoration plantings facing this problem may be direct seeding and/or planting of large-seeded tree species capable of growing past the ferns and shading them out. However, further research with additional tree species is required to ascertain how an understory layer of *N. brownii* affects species of different seed size and life history.

Conclusion

Nephrolepis brownii suppressed tropical forest succession in our tropical restoration plantings experiment. The fern invasion was most successful in unplanted control plots, highlighting the importance of tree canopy cover. In the animal-dispersed treatment where recruiting species richness was highest, there was also a negative correlation between frond density and tree-seedling recruitment. Our seed addition experiment confirmed that this inverse relationship was not simply correlative but caused by the ferns themselves. Future studies should evaluate how restoration plantings can be designed to make them less likely to develop recalcitrant understory layers. While fences cannot be removed to allow ungulate grazers in, we suggest testing the effects of (1) planting an assemblage of species that cover multiple forest strata and have more permanent tree canopies, (2) supplementing canopy gaps with additional seedlings, and/or (3) expanding the dimensions of the plantings.

Acknowledgements

We thank the staff at the Los Tuxtlas Biological Station for their support and Dario Velasco, Jorge Velasco, and Ivonne Popoca for their field assistance. We are grateful to Santiago Sinaca Colín and Álvaro Campos-Villanueva for assistance identifying tree seedlings. We are also grateful to the members of the Wise Lab at the University of Illinois, Chicago (UIC) for comments on the manuscript and the Golden Generation of Los Tuxtlas for support during the field seasons. We are grateful to Ann Marie Gawel, Dalva M. Silva Matos, Cristina Martinez-Garza, and Elizabeth Wandrag for constructive comments on the manuscript and Aaron Goodman for advice on statistical analyses and approaches. This study was supported by two Elmer Hadley Graduate Research Grants from the Department of Biological Sciences at UIC. The restoration plantings were established and maintained with funds from the United States National Science Foundation (DEB 0516259), the University of Illinois, and the National Geographic Society (9302-13). The authors declare no competing interests.

References

Aide TM, Zimmerman JK, Pascarella JB, Rivera L, Marcano-Vega H (2000) Forest regeneration in a chronosequence of tropical abandoned pastures: amplications for restoration acology. Restoration Ecology 8: 328–338. https://doi.org/10.1046/j.1526-100x.2000.80048.x

Allison SD, Vitousek PM (2004) Extracellular Enzyme Activities and Carbon Chemistry as Drivers of Tropical Plant Litter Decomposition1. Biotropica 36(3): 285–296. https://doi.org/10.1646/03180

Barton MK (2015) MuMIn: MuMIn: Multi-Model Inference. R package version 1.43.17. http://cran.r-project.org/package=MuMIn

Bates D, Machler MM, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using {lme4}. Journal of statistical software 67: 1–48. https://doi.org/10.1007/978-94-009-4812-9_8

Beltrán LC, Howe HF (2019) The frailty of tropical restoration plantings. Restoration Ecology 28: 16–21. https://doi.org/10.1111/rec.13066

CABI (2019) Invasive Species Compendium. http://www.cabi.org/isc

de la Peña-Domene M, Martínez-Garza C, Howe HF (2013) Early recruitment dynamics in tropical restoration. Ecological Applications 23: 1124–1134. https://doi.org/10.1890/12-1728.1

de la Peña-Domene M, Minor ES, Howe HF (2016) Restored connectivity facilitates recruitment by an endemic large-seeded tree in a fragmented tropical landscape. Ecology 97: 2511–2517. https://doi.org/10.1002/ecy.1459

den Ouden J (2000) The Role of Bracken (*Pteridium aquilinum*) in forest dynamics. Wageningen, Netherlands: Wageningen University.

Douterlungne D, Levy-Tacher SI, Golicher DJ, Dañobeytia FR (2010) Applying Indigenous Knowledge to the Restoration of Degraded Tropical Rain Forest Clearings Dominated by Bracken Fern. Restoration Ecology 18: 322–329. https://doi.org/10.1111/j.1526-100X.2008.00459.x

Douterlungne D, Thomas E, Levy-Tacher SI, Paynter Q (2013) Fast-growing pioneer tree stands as a rapid and effective strategy for bracken elimination in the Neotropics. Journal of Applied Ecology: 1257–1265. https://doi.org/10.1111/1365-2664.12077

Foster S, Janson CH (1985) The Relationship between Seed Size and Establishment Conditions in Tropical Woody Plants. Ecology 66: 773–780. https://doi.org/10.2307/1940538

George LO, Bazzaz FA (1999a) The Fern Understory as an Ecological Filter: Emergence and Establishment of Canopy-Tree Seedlings. Ecology 80: 833–845. https://doi.org/10.1890/0012-9658(1999)080[0833:TFUAAE]2.0.CO;2

George LO, Bazzaz FA (1999b) The Fern Understory as an Ecological Filter: Growth and Survival of Canopy-Tree Seedlings. Ecology 80: 846–856. https://doi.org/10.1890/0012-9658(1999)080[0846:TFUAAE]2.0.CO;2

González-Soriano E, Dirzo R, Vogt RC (1997) Historia natural de los Tuxtlas. Universidad Autonoma de Mexico (UNAM), Instituto de Biologia, Instituto de Ecología, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Mexico.

Hill JD, Silander JA (2001) Distribution and dynamics of two ferns: *Dennstaedtia punctilobula* (Dennstaedtiaceae) and *Thelypteris noveboracensis* (Thelypteridaceae) in a Northeast mixed hardwoods-hemlock forest. American Journal of Botany 88: 894–902. https://doi.org/10.2307/2657041

Holl KD, Quiros-Nietzen E (1999) The effect of rabbit herbivory on reforestation of abandoned pasture in southern Costa Rica. Biological Conservation 87: 391–395. https://doi.org/10.1016/S0006-3207(98)00051-2

Horsley SB (1993) Mechanisms of interference between hay-scented fern and black cherry. Canadian Journal of Forest Research 23: 2059–2069. https://doi.org/10.1139/x93-257

Hovenkamp PH, Miyamoto F (2005) A conspectus of the Native and Naturalized Species of *Nephrolepis* (Nephrolepidaceae) in the World. Blumea – Biodiversity, Evolution and Biogeography of Plants 50: 279–322. https://doi.org/10.3767/000651905X623003

Ibarra-Manríquez G, Martínez-Morales M, Cornejo-Tenorio G (2015) Frutos y semillas del bosque tropical perennifolio: región de Los Tuxtlas, Veracruz. Conabio, México, 348 pp.

Ibarra-Manríquez G, Sinaca Colín S (1995) Lista florística comentada de la Estación de Biología Tropical "Los Tuxtlas", Veracruz, México. Revista de Biología Tropical: 75–115.

Jakobs G, Weber E, Edwards PJ (2004) Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. Diversity and Distributions 10: 11–19. https://doi.org/10.1111/j.1472-4642.2004.00052.x

Kuznetsova A, Brockhoff PB, Christensen RH (2017) lmerTest package: tests in linear mixed effects models. Journal of statistical software 82: 1–26. https://doi.org/10.18637/jss.v082.i13

Keane R, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Lamb D, Erskine PD, Parrotta JA (2005) Restoration of degraded tropical forest landscapes. Science 310: 1628–1632. https://doi.org/10.1126/science.1111773

Laurance WF (2008) Theory meets reality: How habitat fragmentation research has transcended island biogeographic theory. Biological Conservation 141: 1731–1744. https://doi.org/10.1016/j.biocon.2008.05.011

Longworth JB, Williamson GB (2018) Composition and Diversity of Woody Plants in Tree Plantations Versus Secondary Forests in Costa Rican Lowlands. Tropical Conservation Science 11: 1–13. https://doi.org/10.1177/1940082918773298

Lüdecke D (2019) Sjstats: Collection of Convenient Functions for Common Statistical Computations. R package version 0.18.0. https://CRAN.R-project.org/package=sjstats

Magnago LFS, Rocha MF, Meyer L, Martins SV, Meira-Neto JAA (2015) Microclimatic conditions at forest edges have significant impacts on vegetation structure in large Atlantic forest fragments. Biodiversity and Conservation 24: 2305–2318. https://doi.org/10.1007/s10531-015-0961-1

Marrs RH, Johnson SW, Duc MGL (2008) Control of bracken and restoration of heathland. VIII. The regeneration of the heathland community after 18 years of continued bracken control or 6 years of control followed by recovery. Journal of Applied Ecology 35: 857–870. https://doi.org/10.1111/j.1365-2664.1998.tb00004.x

Mendoza E, Fay J, Dirzo R (2005) A quantitative analysis of forest fragmentation in Los Tuxtlas, southeast Mexico: patterns and implications for conservation. Revista chilena de historia natural 78(3): 451–467. https://doi.org/10.4067/S0716-078X2005000300008

Parrotta JA, Knowles OH (2001) Restoring tropical forests on lands mined for bauxite: Examples from the Brazilian Amazon. Ecological Engineering 17: 219–239. https://doi.org/10.1016/S0925-8574(00)00141-5

Popoca-Ortega L (2016) Lluvia de semillas en parcelas de restauración ecológica en la selva tropical de Los Tuxtlas, Veracruz, México. Tesis de Licenciatura, Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos. Cuernavaca, Morelos, México.

Proctor GR (1989) Ferns of Puerto Rico and the Virgin Islands. Memoirs of the New York Botanical Garden 53: 1–389. https://doi.org/10.2307/1547180

Rey Benayas JM, Bullock JM, Newton AC (2008) Creating woodland islets to reconcile ecological restoration, conservation, and agricultural land use. Frontiers in Ecology and the Environment 6: 329–336. https://doi.org/10.1890/070057

Robinson RC, Sheffield E, Sharpe JM, Mehltreter K, Walker LR, Sharpe JM (2010) Problem ferns: their impact and management. Fern Ecology: 255–322. https://doi.org/10.1017/CBO9780511844898.009

Royo AA, Carson WP (2006) On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. Canadian Journal of Forest Research 36: 1345–1362. https://doi.org/10.1139/x06-025

Sansevero JBB, Prieto PV, de Moraes LFD, Rodrigues PJP (2011) Natural Regeneration in Plantations of Native Trees in Lowland Brazilian Atlantic Forest: Community Structure, Diversity, and Dispersal Syndromes. Restoration Ecology 19: 379–389. https://doi.org/10.1111/j.1526-100X.2009.00556.x

Sharpe JM, Shiels AB (2014) Understory fern community structure, growth and spore production responses to a large-scale hurricane experiment in a Puerto Rico rainforest. Forest Ecology and Management 332: 75–86. https://doi.org/10.1016/j.foreco.2014.01.023

van Schaik CP, Terbourgh JW, Wright SJ (1993) The Phenology of Tropical Forests: Adaptive Significance and Consequences for Primary Consumers. Annual Review of Ecology and Systematics 24: 353–377. https://doi.org/10.1146/annurev.es.24.110193.002033

Valencia-Esquivel I (2012) Efecto de plantaciones de restauración ecológica experimental en la caída de hojarasca en un pastizal tropical lluvioso en Los Tuxtlas, Veracruz. PHD Thesis. Universidad Autónoma del Estado de Morelos.

Wunderle JM (1997) The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. Forest Ecology and Management 99: 223–235. https://doi.org/10.1016/S0378-1127(97)00208-9

Young TP, Peffer E (2010) "Recalcitrant understory layers" revisited: arrested succession and the long life-spans of clonal mid-successional species. Canadian Journal of Forest Research 40: 1184–1188. https://doi.org/10.1139/X10-066

Supplementary material 1

Model output for canopy cover characteristics by restoration treatment

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: Statistics table

Explanation note: Mixed-effect model output for canopy openness (100 – Canopy Cover %) and frequency of planted trees providing canopy cover by restoration treatment. Canopy openness model was built using a negative binomial distribution.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl1

Supplementary material 2

Trees by origin and restoration treatment

Authors: Luis C. Beltrán

Data type: Count

Explanation note: Total number of trees (>2m) per treatment, including both, recruited and planted trees. This figure comes from a separate unpublished study carried out by Luis C. Beltrán.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl2

Supplementary material 3

Information on planted tree species

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: Descriptive

Explanation note: Family, life history, dispersal mode, height range, and forest strata occupied by the planted species in the experimental restoration plantings. Height ranges from Ibarra-Manríquez et al. (2015) and Ibarra-Manríquez and Sinaca Colín (1995). Strata classified from published height ranges.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl3

Supplementary material 4

Fern data from quadrat study

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: count, density, percent, measurement, and species composition

Explanation note: This table contains the data from the 384 quadrats where we determined: frond density, frond length, canopy cover, the identity of species covering the quadrat with their canopy, and the percent understory cover of grass or lianas.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl4

Supplementary material 5

Recruit data from quadrat study

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: occurrence

Explanation note: This table includes the occurrence of each plant (>10 cm tall) that we found in our study's quadrats. The life history and dispersal mode of each species is presented.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl5

Supplementary material 6

Seed addition experiment data

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: Count and measurements

Explanation note: This table contains the results from the seed addition experiment. The shadehouse treatment corresponds to seeds sown in a shadehouse to confirm seed viability (not included in analysis). The columns for each month correspond to height measurements taken monthly. Plant biomass measurements are of dry mass (g).

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl6

Supplementary material 7

Output from mixed-effects models on frond density

Authors: Luis C. Beltrán, Karla María Aguilar-Dorantes, Henry F. Howe

Data type: Statistics table

Explanation note: Summary from the mixed-effects models used in this study including: (1) Frond Density x Treatment, (2) Frond Length x Treatment, (3) Frond Density x Frond Length, and (4) Frond Density x Canopy Cover.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.59.51906.suppl7